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EFFECT OF SUPERHIGH-FREQUENCY ELECTROMAGNETIC RADIATION ON ELECTROPHORETIC MOBILITY OF ERYTHROCYTES

[Text] Changes in electrophoretic mobility (EM) have been demonstrated in human erythrocytes under the influence of superhigh-frequency (SHF) electromagnetic radiation in the 1009 MHz range; they are related to the duration and intensity of irradiation. These changes are attributable to two causes: deformation of the double electrical layer and structural changes in the erythrocyte membrane, which are the consequence of phasic change in structured [cross-linked] membrane fluid into a more liquid state. The EM changes are reversible.

With each year, there is an appreciable increase in amount of research dealing with biological activity of SHF electromagnetic radiation. This is due to the need to protect people from occupational and nonoccupational exposure to SHF fields, as well as the desire to upgrade SHF methods that are used in biology and medicine. The experimental data indicate that SHF radiation, of both thermal and nonthermal intensity, elicits numerous changes in various tissues and organs of man and animals [1, 2]. The effect of SHF waves is manifested in offspring [3].

At the same time, there are still very few studies being pursued for demonstration of the primary mechanisms of the observed effects of SHF. With reference to the physicochemical mechanisms of biological activity of SHF waves, the potential change in structure and function of cell membranes under the influence of SHF radiation should be considered one of the focal questions. We previously obtained direct experimental data on the effects of SHF waves on permeability of human erythrocyte membranes to potassium and sodium ions [4]. Exposure of a suspension of erythrocytes to 45 mW/cm^3 SHF radiation in the range of 1009 MHz elicited an increase in amount of potassium ions and decrease in sodium ions in the incubation medium, as compared to the control, i.e., there was a decrease in the concentration

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gradient of these ions on the membrane. Exposure of erythrocyte suspension to SHF waves combined with monoiodoacetate led to faster diffusion of potassium and sodium through the membrane. Shtemler [5] has also demonstrated a change in transport of these ions in human erythrocytes under the influence of SHF waves. We should mention the recently demonstrated effect of decimeter radiowaves on the membrane of rabbit liver mitochondria, as manifested by the dissociating effect of SHF waves on oxidative phosphorylation [6].

As we know, the membrane structure determines its surface charge, on which electrophoretic mobility (EM) of cells depends, at a given temperature, pH and with unchanged ionic force and viscosity of the ambient solution. The objective of the work in question was to determine the EM of erythrocytes under the influence of UHF radiation varying in intensity and duration.

Methods and Techniques

A suspension of stored human erythrocytes was prepared by three-fold removal of plasma in isotonic phosphate buffer with glucose (pH 7.4), where the cell content constituted about 5 million ml. They were exposed to 1009 MHz microwaves in a coaxial cell at a constant temperature (37°C) using a special device that has been described previously [7]. Control suspensions of erythrocytes were kept at 37°C in an incubator during the irradiation period. After irradiation, the erythrocyte suspension was diluted 10^3-fold in phosphate buffer, and EM of cells was determined in a horizontal capillary by the method of microelectrophoresis. The techniques for electrophoresis of blood are described in the monograph of Kharmonenko and Rakityanskaya [8]. We determined EM immediately after irradiation, then every 10 min for 90 min.

Results

The EM of intact erythrocytes (control) are rather stable in the course of the observation period (90 min) and are in the range of $1.28 \pm 0.03 \pm 1.33$ $0.04 \cdot 10^{-4}$ cm^2 V^{-1}s^{-1}, which is consistent with the literature [9]. The EM of erythrocytes exposed to 45 mW/cm SHF for 30 min is higher immediately after exposure, and it constitutes $1.45 \pm 0.03 \cdot 10^{-4}$cm^2 V^{-1} s^{-1}. This is followed by a phase of decline. In the 60th min, EM reaches the control level. Thereafter, there is a decline of EM to $1.22 \pm 0.02 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}, which is appreciably lower than in the control, and then a rise to the control level by the 80th min.

Figure 1 illustrates EM changes in irradiated erythrocytes during and after irradiation.

Table 1 lists the values of EM of irradiated erythrocytes as function of duration of SHF irradiation at an intensity of 45 mW/cm^3. The data in this table indicate that the general changes in EM of erythrocytes during and after 15-min exposure to SHF are the same as in the case of 30-min exposure. However, with regard to absolute change in EM in the positive direction, as compared to the control, it is less marked for the first 50 min of observation. In addition, there is virtually no decline of EM, as compared

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to the control in the 70th min. A reduction of exposure to SHF to 8 min results in a normal EM of erythrocytes immediately after irradiation. It then increases, reaching a maximum by the 30th min, then decreases again to the control level. Here, too, there is no phase of decline of EM in the 70th min, as compared to the control.
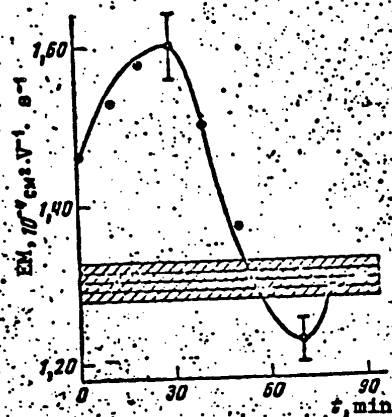

Figure 1.
Change in electrophoretic mobility of erythrocytes as function of time, following 30-min exposure to 45 mW/cm³ SHF. The striped area refers to EM of intact erythrocytes.

X-axis, time after irradiation (t, min).

Table 1. Electrophoretic mobility of erythrocytes ($10^{-4} cm^2 V^{-1} s^{-1}$) with exposure to 45 mW/cm³ SHF, at different times thereafter

Interval between irradiation & EM reading, min	Exposure time, min		
	15	8	4
0	1.33±0.03	1.30±0.04	1.20±0.02
10	1.46±0.04	1.40±0.02	1.32±0.04
20	1.51±0.02	1.43±0.03	1.37±0.03
30	1.54±0.03	1.46±0.03	1.33±0.02
40	1.45±0.02	1.33±0.02	1.34±0.04
50	1.36±0.04	1.31±0.04	1.29±0.03
60	1.31±0.03	1.27±0.02	1.31±0.03
70	1.25±0.02	1.23±0.04	1.32±0.02
80	1.30±0.04	1.32±0.03	—
90	1.31±0.03	1.29±0.03	—

The values of EM of erythrocytes after exposure to SHF for 4 min are of interest: Immediately after exposure, the EM of irradiated erythrocytes is lower than in the control. It then increases and is higher than the control in the 30th min of observation, then drops to virtually the control level by the 40th min.

Thus, we observe dissimilar changes in EM of erythrocytes, and even changes in different directions, depending on the dosage of SHF radiation. Such

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changes can be explained on the basis of the assumption that they are induced
by two superimposed processes: deformation of the double electrical layer
(DEL) on the cell surface and some possible structural changes in the membrane
proper, leading to a change in amount of potential-forming ionizing groups on
its surface. The former process leads to compression of DEL and, accordingly,
a decrease in EM of erythrocytes. The latter process, conversely, elicits
appearance on the membrane surface of an additional amount of potential-forming
ions and, as a result, increase the EM of the cells. Both processes are
reversible. The thickness of the DEL is restored sooner (20-30 min after
discontinuing irradiation). At first, the drop of surface charge of erythro-
cytes proceeds slowly and then, after the 30th min, rather rapidly, so that
EM is normalized within 50-60 min after exposure to SH. Figure 2 illustrates
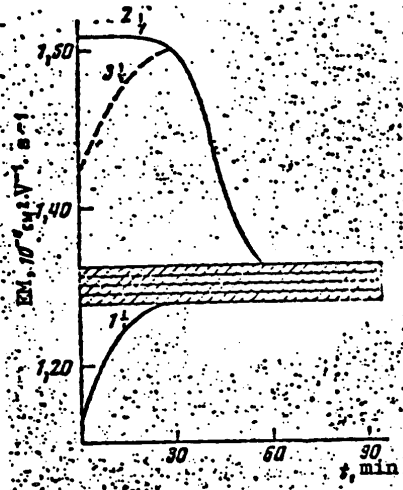the curve of EM as function of postradiation time, which is plotted in two
components.

Figure 2.
Separation of EM—postradiation time
(curve 3) into two components:
1) curve of EM change due to de-
formation of DEL; 2) curve of EM
change due to possible structural
changes in the membrane. Striped
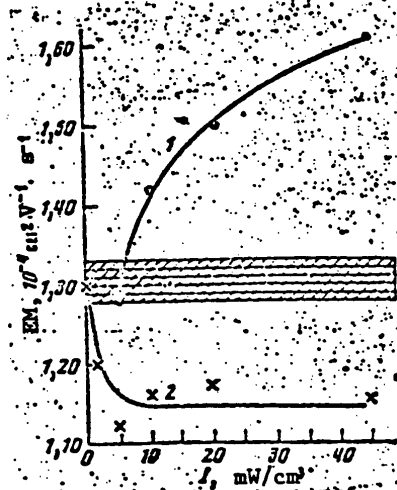area refers to EM values for
intact erythrocytes

Figure 3.
Change in EM of erythrocytes due to
deformation of DEL (curve 2) and
possible structural changes in the
membrane (curve 1) as function of
intensity of SHF radiation. Striped
area refers to EM values for intact
erythrocytes. X-axis, intensity of
SHF radiation (I, mW/cm^3)

The small phase of decrease of EM, as compared to the control, in the 70th
min of observation, demonstrated in the case of 30-min exposure to SHF, is
attributable, in our opinion, to regulator processes in the cell membrane,
which lead to restoration of normal EM. In the case of substantial

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changes, restoration of normal charge of the membrane surface occurs under over-regulation, and this elicits the EM change in question.

Curves 1 and 2, and Table 1 also indicate that deformation of DEL and the change in charge of the surface of erythrocyte membranes are dissimilarly related to SHF dosage. For better demonstration of this function we studied the dynamics of change in EM of erythrocytes as function of time after exposure to SHF radiation varying in intensity but for the same period of time (30 min). The results obtained are listed in Table 2.

Table 2. EM of erythrocytes (10^{-4} cm^2 $V^{-1}s^{-1}$) in the case of exposure to different intensities of SHF radiation for 30 min

Interval between end of irradiation and EM reading, min	Radiation intensity, mW/cm³			
	20	10	5	2
0	1,34±0,02	1,26±0,04	1,12±0,03	1,29±0,02
10	1,41±0,04	1,32±0,04	1,22±0,02	1,27±0,04
20	1,44±0,03	1,37±0,02	1,27±0,04	1,31±0,03
30	1,47±0,03	1,40±0,03	1,30±0,02	1,30±0,03
40	1,35±0,02	1,34±0,02	1,29±0,04	1,32±0,03
50	1,31±0,02	1,31±0,03	1,28±0,03	—
60	1,29±0,03	1,31±0,03	—	—
70	1,25±0,02	1,29±0,03	—	—
80	1,29±0,04	1,32±0,02	—	—
90	1,32±0,03	—	—	—

The EM changes as function of time can be explained, in this instance too, from the standpoint of the existence of two SHF effects in different directions, which we mentioned above.

On the assumption that, for the first 20-30 min, the change in EM as function of time is due mainly to restoration of initial DEL, the following can be estimated from the data in Table 2: deformation (compression) of DEL under the influence SHF waves depends little on intensity of radiation and has a threshold in the range of 1-3 mW/cm³. The decrease in EM of cells induced by reduction [compression] of DEL constitutes 0.14÷0.18·$10^{-4}cm^2$ $V^{-1}s^{-1}$. The second process, the change in charge of the membrane surface, is directly related to intensity of the SHF field, and it is virtually undemonstrable at low intesities (less than 5-7 mW/cm³). With increase in intensity, EM first increases more rapidly, then more slowly, presenting a tendency toward saturation. Figure 3 illustrates changes in EM as function of deformation of DEL and possible structural changes in erythrocyte membranes.

Discussion

Two main processes occur in a biological medium under the influence of SHF fields: relaxation oscillations of dipole molecules of water causing dieletric loss of SHF energy, and oscillations of free charges, which elicit loss of conduction. As a result, SHF energy is transformed into thermal energy and raises the temperature of the medium. Depending on the microwave

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frequency, the share of each of these types of loss varies, since dielectric loss increases with increase in frequency. If the biological medium were homogeneous, the only effect of SHF radiation should be to lower the temperature of the system, i.e., it would be a thermal effect. But, in actuality, the situation is more complicated.

Thanks to the research of recent years, the important role of hydrophobic interactions has been demonstrated in stabilization of structures of the cell membrane [10, 11]. Evidently, with a change in degree of structurization and amount of structurized water, the membrane becomes destablized and protein-lipid interaction will be impaired.

As far back as the 1950's [12, 13], it was shown that the characteristic frequency of structurized fluid is in the SHF range. In such water, there will be more dielectric loss of SHF energy than in ordinary water. Most probably, these losses will induce phasic change in structurized water and, accordingly, could lead to conformational changes in the membrane macromolecules, which we have already reported [14]. At the same time, the concentration of ions in the first layer of DEL gegenions [counterions] is appreciably higher in the immediate vicinity of the membrane surface than in the rest of the extracellular and intracellular fluid. Consequently, the relative share of loss of conduction would also be higher near the membrane surface. On the whole, both near the surface and in the membrane proper, considerably more SHF energy will be absorbed than the average for the entire medium. This energy is partially dissipated, leading to a general change in temperature of the system, while part is expended for destruction of hydrate membranes of the ionized membrane surface, as well as phasic change in structurized "hydrophobic" water within the membrane into a more liquid state.

In erythrocytes, the surface charge of membranes is determined primarily by the rather markedly ionized phosphate groups of oriented polar molecules of phospholipids, which form the lipid layer, as well as ionized groups of proteins. The partially negative charges of phosphate groups are shielded by the positively charged groups of protein molecules. The extent of shielding depends on protein-lipid interaction. Consequently, the surface charge of the membrane is a function of two interrelated factors: conformation of protein molecules and extent of protein-lipid interaction, from the standpoint of reciprocal orientation of their ionized groups. In our opinion, compression of erythrocyte DEL occurs as a result of "melting" of the hydrate membranes of ionized groups on the membrane, while the change in charge of the membrane surface results from possible conformational changes in protein molecules and impairment of protein-lipid interaction. Both these processes are reversible under ordinary conditions.

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BIBLIOGRAPHY

1. Presman, A. S., "Electromagnetic Fields and Living Nature," Moscow, "Nauka," 1968.

2. Minin, B. A., "Ultrahigh-Frequency Waves and Safety for Man," "Sov. radio," Moscow, 1974.

3. Scott, J., MICROWAVE J., No 1, 9, 1971.

4. Ismailov, E. Sh., NAUCHNYYE DOKLADY VYSSHEY SHKOLY, BIOL. NAUKI [Scientific Papers of Higher Educational Institutions, Biological Sciences], 3, 58, 1971.

5. Shtemler, V. M. in: "Gigiyena truda i biologicheskoye deystviye elektromagnitnykh voln radiochastot" [Industrial Hygiene and Biological Effects of Radio-Frequency Electromagnetic Waves], Moscow, 63, 1972.

6. Zubkova, S. M.; Zhuravlev, A. I.; and Grigor'yeva, V. D. Ibid, 68, 1972.

7. Ismailov, E. Sh. in: "Voprosy fiziologii, biokhimii, zoologii i parazitologii" [Problems of Physiology, Biochemistry, Zoology and Parasitology], Makhachkala, 4, 90, 1970.

8. Kharamonenko, S. S., and Rakityanskaya, A. A. "Electrophoresis of Blood Cells Under Normal and Pathological Conditions," Minsk, "Belarus'," 1974.

9. Ponder, E., and Ponder, R. V. NATURE, 197, 4863, 178, 1963.

10. Borovyagin, V. L. "Cell Membranes," BIOFIZIKA [Biophysics], 16, 4, 746, 1971.

11. Singer, S. J., ANN. REV. BIOCHEM., 43, 805, 1974.

12. Schwan, H. ADV. BIOL. AND MED. PHYS., 5, 147, 1957.

13. Shvan, G., and Gogel'khut, P. in: "SHF Energetics," Moscow, "Mir," 3, 33, 1971.

14. Ismailov, E. Sh. "IV Mezhdunar. biofizich. kongress, tez. sekts. soobshch." [Fourth International Biophysical Congress, Summaries of Section Papers], Moscow, 4, 434, 1972.

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